Journal of Magnetism and Magnetic Materials 00 (2008) 000–000

www.elsevier.com/locate/immm

Functional Magnetic Nanoparticles for Medical Application

Yuko Ichiyanagi[a]*, Shinji Moritake[a], Shu Taira[b], Mitsutoshi Setou[b]

[1]*Graduate School of Engineering, Department of Physics, Yokohama National University, Yokohama 240-8501 Japan*
Mitsubishi Kagaku Institute of Life Sciences, Molecular Gerontology Research Group, Tokyo 194-8511 Japan

Abstract

We prepared an amino-substituted nanoparticle by means of the amino-silane coupling procedure. The original magnetic particles were γ-Fe_2O_3, which ranged in size from 1.3 to 34 nm, surrounded by amorphous SiO_2. The modification of the magnetic particle by the addition of an amino group was confirmed using a Fourier transform infrared spectrophotometer (FT-IR). The X-ray diffraction patterns showed a spinel structure both before and after modification of the amino group. The magnetization curve indicated paramagnetic behavior for the 3-nm particles, superparamagnetic behavior for the 7-nm particles, and ferromagnetic behavior for 9-nm particles at room temperature. A fluorescent reagent was applied to the particle, and the particle was introduced into a cell. The magnetic particles in the cell were localized using an external magnetic field.

PACS: 87.90.+y; 87.15.Da

Keywords: nanomedicine; magnetic nanoparticle; drug delivery system

1. Introduction

Magnetic nanoparticles (MNPs) have attracted significant interest, as they have some peculiar characteristics, such as quantum size effects and magnetic quantum tunneling. The future applications of MNPs are expected to be many, not only in high-density magnetic recording, but also as a marker or labeling material in the medical field, whereby MNPs are linked to DNA, antibodies, and other biological molecules.

Previously, we have prepared several types of MNPs encapsulated in amorphous SiO_2, and studied their magnetic properties [1,2,3]. Attempts to introduce magnetic particles into living cells are beginning to bear fruit, especially for drug delivery systems [4,5]. However, most of the MNPs used to date have been coated with cationic reagents, since the surfaces of target cells are negatively charged. It is remarkable that the magnetic particles in the present study are only several nanometers in size, and that they are able to enter target cells without cationic coating. For biological applications, it is very important to conjugate MNPs with biological molecules, such as amino groups. The MNPs used in the present study were surrounded by amorphous SiO_2, i.e., Si ions were located on the outer surface. This characteristic structure enables amino-silane coupling. In the present study, we selected γ-Fe_2O_3 nanoparticles because of their strong magnetization, even at room temperature.

2. Experimental

The γ-Fe_2O_3 MNPs surrounded by amorphous SiO_2 (Fe-MNPs) were prepared by mixing aqueous solutions of $FeCl_2 \cdot 4H_2O$ and $Na_2SiO_3 \cdot 9H_2O$ at pH 7.0 [1]. The obtained sample was identified as γ-Fe_2O_3 (maghemite) surrounded by amorphous SiO_2, and the particle sizes were found to range from 1.3 nm to 34 nm, based on powder X-ray diffraction patterns [3]. The Fe-MNPs were functionalized by washing with EtOH and ultrapure water, centrifuging for 3 minutes, and removal with a pipette. 3-Aminopropyltriethoxysilane (γ-APTES) was used for the conjugation of the Fe-MNPs with amino groups. The mixture was stirred for 10 minutes, heated to 403 K, and then washed several times with water. The functionalized MNPs were labeled with rhodamine, which is a fluorescent material. The labeled MNPs were introduced into

* Corresponding author. Tel.: +81-45-339-4185; fax: +81-45-339-4185.
E-mail address: yuko@ynu.ac.jp.

PTK2 cells, as well as into the ear of a mouse; wherein they were detected ear using a button magnet with a surface flux density of 240 mT. The magnetization measurements for the MNPs were performed with a SQUID magnetometer (MPMS; Quantum Design) under a ±50 kOe field.

4. Results and Discussion

Magnetization Figure 1 shows the magnetization curve for the 7-nm γ-Fe_2O_3 MNPs at room temperature. Superparamagnetic behavior was observed, with a large magnetization value of 0.2 μ_B per Fe ion. After silanization, the magnetic properties and X-ray patterns were largely unchanged. Although the level of magnetization was reduced by 20% after silanization compared to before silanization, it was still sufficiently large. For a particle size of 7 nm, the formation of a single domain would be expected. The magnetic properties of this particle are useful for designing drug delivery systems and for compound purification. Ferromagnetic behavior was also observed for the 9-nm sample.

FT-IR measurement Fourier transform infrared spectroscopy (FT-IR) was performed to confirm the conjugation of the amino group with the magnetic particle, as shown in Figure 2. The typical O-H peak (3500-3900 cm^{-1}) and the N-H amino group peak (2982-2822 cm^{-1}) were detected in the spectrum of the functionalized Fe-MNPs, while only the O-H peak was observed in the spectrum of the unmodified Fe-MNPs.

Thus, the Fe-MNPs were successfully functionalized with amino groups via silanization, which facilitates the attachment of these particles to other biological molecules.

Insertion into living cells We introduced some of the 3-nm γ-Fe_2O_3 MNPs into living cells. Labeled Fe-MNPs in pure water were dropped onto a culture of growing PTK2 cells. Following the uptake of Fe-MNPs, a TEM image of the intracellular space was obtained. Large, high-density spots of around 20 nm in size were detected, and they were clearly distinguishable from ribosomes.

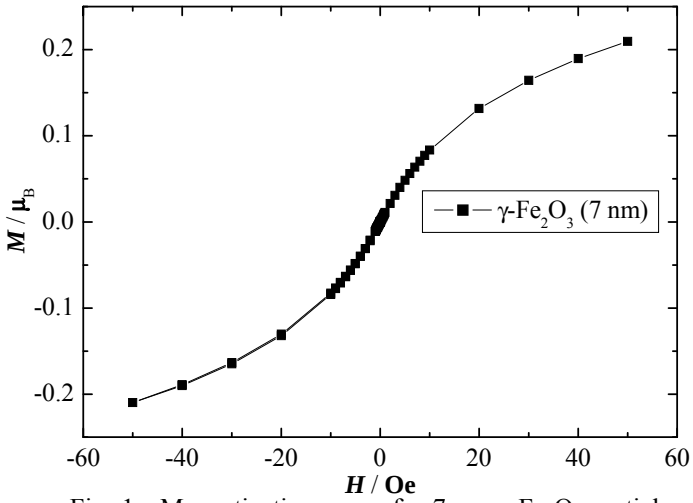

Fig. 1. Magnetization curve for 7-nm γ-Fe_2O_3 particles at room temperature.

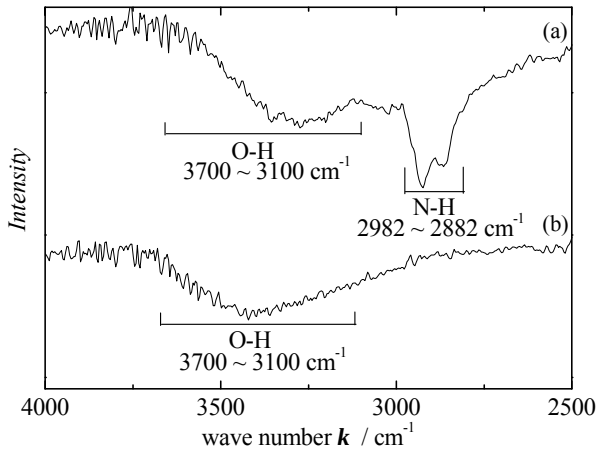

Fig. 2. FT-IR spectra of the functionalized magnetic nanoparticles (upper) and the original nanoparticles (lower).

Concentration by magnetic force A button magnet was attached to the ear of a mouse, while labeled MNPs were applied with brush to the other side of the ear. After 24 hours, cells from sections of the ear were observed using a confocal laser scanning microscope (LSMSPASCAL; Carl Zeiss). Strong fluorescence was observed within the area circumscribed by a radius of approximately 2.5 mm from the point at which the button magnet was positioned. The labeled MNPs penetrated the dermis, hypodermis, and muscle, and reached the cartilage.

To summarize, the Fe-MNPs were modified by the addition of amino groups, labeled with a fluorescent material, introduced into living cells, and localized in vivo by means of an external magnetic field. It is remarkable that the MNPs in the present study could be introduced into living cells without cationic coating. This is probably due to the extremely small size of the particles.

In conclusion, novel functional magnetic nanoparticles were successfully developed, and these nanoparticles are expected to become useful in future medical applications.

Acknowledgement

This work was partially supported by a Grand-in-Aid for Scientific Research (no. 15510089) from the Japan Society for the Promotion of Science.

References

[1] Y. Ichiyanagi et al. *Trans. Mater. Res. Sci. J*, **26** (2001), p. 1097.
[2] Y. Ichiyanagi et al. *J. Magn. Magn. Mater.* **272-276** (2004), p. e1245.
[3] Y. Ichiyanagi et al. *J. Ther. Anal. Calor.* 69 (2002), p. 919.
[4] A.K. Gupta, S. Wells, *IEEE Trans Nanobiosci*. **3** (2004), p.66.
[5] A. Ito, M. Shinkai, H. Honda, T. Kobayashi, *J. Bio. Bioeng*. **100(1)** (2005), p. 1.